Journal of Cell Science 113, 207-214 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS1044

The PI 3-kinase isoforms p110α and p110β have differential roles in PDGF- and insulin-mediated signaling

Roya Hooshmand-Rad[1,*], Lucie Hájková[2], Peter Klint[4], Roger Karlsson[2], Bart Vanhaesebroeck[3], Lena Claesson-Welsh[4] and Carl-Henrik Heldin[1,‡]

[1]Ludwig Institute for Cancer Research, BMC, Box 595, S-751 24 Uppsala, Sweden
[2]Dept of Cell Biology, WGI, Stockholm University, S-106 91 Stockholm, Sweden
[3]Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, UK
[4]Dept of Medical Biochemistry and Microbiology, BMC, Box 575, S-751 23 Uppsala, Sweden
*Present address: Burnham Institute for Cancer Research, 1091 North Torvey Pines Road, La Jolla, CA 92039, USA
‡Author for correspondence

Accepted 4 November 1999; published on WWW 13 January 2000

SUMMARY

Phosphoinositide 3′-kinases constitute a family of lipid kinases implicated in signal transduction through tyrosine kinase receptors and heterotrimeric G protein-linked receptors. Phosphoinositide 3′-kinases that bind to the platelet-derived growth factor receptor are composed of two subunits: the p85 subunit acts as an adapter and couples the catalytic p110 subunit to the activated receptor. There are different isoforms of p85 as well as of p110, the individual roles of which have been elusive. Using microinjection of inhibitory antibodies specific for either p110α or p110β we have investigated the involvement of the two p110 isoforms in platelet-derived growth factor- and insulin-induced actin reorganization in porcine aortic endothelial cells. We have found that antibodies against p110α, but not antibodies against p110β, inhibit platelet-derived growth factor-stimulated actin reorganization, whereas the reverse is true for inhibition of insulin-induced actin reorganization. These data indicate that the two phosphoinositide 3′-kinase isoforms have distinct roles in signal transduction pathways induced by platelet-derived growth factor and insulin.

Key words: PI3-kinase, Cytoskeleton, PDGF, Insulin

INTRODUCTION

Phosphoinositide 3′-kinase (PI3-K) activity induced by growth factor activation of several cell surface receptors leads to the production of the membrane lipids phosphatidylinositol-3-phosphate (PtdIns(3)P), PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. PI3-K is a heterodimer consisting of a regulatory and a catalytic subunit. Recently several isoforms of both the regulatory and catalytic subunits of PI3-K have been identified. The catalytic subunits p110 α, β, and δ have been shown to be associated with p85α, p85β and p55γ adapter proteins which mediate activation by protein tyrosine kinase receptors (Hiles et al., 1992; Hu et al., 1993; Vanhaesebroeck et al., 1997b). The p110γ isoform, in contrast, binds to a p101 adapter protein and is associated with and activated by βγ subunits of G proteins (Stephens et al., 1994; Stoyanov et al., 1995). The p110β subunit has also been found to be activated by the βγ subunits of G proteins (Kurosu et al., 1997). The p110α and p110β isoforms are both widely expressed whereas the p110δ subunit is predominantly expressed in haematopoietic cells. The p85 subunit contains a wide range of domains involved in protein-protein interactions (Cohen et al., 1995). These include an N-terminal Src homology-3 (SH3) domain, two SH2 domains, two proline-rich regions, a region related to the break point cluster region (*bcr*) gene, and an inter-SH2 domain involved in interaction with p110 and regulation of its kinase activity (Carpenter et al., 1993; Klippel et al., 1994).

Binding of platelet-derived growth factor (PDGF) isoforms to their tyrosine kinase receptors causes receptor dimerization leading to autophosphorylation of the receptors on specific tyrosine residues (for review see Heldin et al., 1998). These phosphorylated tyrosine residues then serve as binding sites for many SH2 domain containing proteins (Claesson-Welsh, 1994), such as the p85 subunit of PI3-K which binds to tyrosine residues 740 and 751 in the PDGF β-receptor (Carpenter et al., 1993; Fantl et al., 1992; Kazlauskas et al., 1992). The binding of p85 to these two tyrosine residues, which are localized in YXXM motifs that are known to be optimal for PI3-K binding, probably leads to an activating conformational change in the catalytic p110 subunit and also brings the PI3-K heterodimer in contact with its lipid substrates.

PI3-K has been implicated as an important mediator of many PDGF-induced cellular responses, such as prevention of apoptosis (Yao and Cooper, 1995), as well as stimulation of mitogenesis (Roche et al., 1994), actin reorganization and chemotaxis (Hooshmand-Rad et al., 1997; Kundra et al., 1994; Nobes et al., 1995; Wennström et al., 1994a,b). PDGF receptor mutants lacking the tyrosines involved in PI3-K binding are

unable to mediate membrane ruffling and chemotaxis. These responses are also inhibited by low molecular mass inhibitors of PI3-K activity such as wortmannin and LY294002. The inhibition of membrane ruffling is overcome by introduction of constitutively active Rac, a member of the Rho family of small GTPases, suggesting that Rac acts downstream of PI3-K in signaling pathways leading to reorganization of actin and chemotaxis (Hooshmand-Rad et al., 1997; Wennström et al., 1994b).

The insulin receptor also belongs to the family of receptor protein tyrosine kinases. Activation of the receptor kinase by ligand binding results in the rapid phosphorylation of substrates, such as insulin receptor substrate-1, to which PI3-K is recruited. PI3-K has been shown to play a major role in insulin-induced actin rearrangements (Kotani et al., 1994), DNA synthesis (Cheatham et al., 1994; Frevert and Kahn, 1997; Jhun et al., 1994; McIlroy et al., 1997), and differentiation (Kaliman et al., 1998; Tomiyama et al., 1995).

In the present study we have investigated the possibility that p110α and p110β have different functions in signaling from the PDGF β-receptor and insulin receptor with respect to actin rearrangement, by analysis of the effects of microinjection into target cells of inhibitory antibodies specific for either p110α or p110β.

MATERIALS AND METHODS

Antibodies

Synthetic peptides corresponding to the 15 C-terminal amino acid residues of p110α and p110β (K M D W I F H T I K Q H A L N and K V N W M A H T V R K D Y R S, respectively) were coupled to keyhole limpet hemocyanin (KLH, Calbiochem). The peptides and KLH were mixed at equal concentrations (0.5 mg/ml) in 0.1 M sodium acetate, pH 5.0, for 12 hours at 4°C. Ethyldimethylcarbodiimid (EDC; Sigma) was added to a final concentration of 20 mg/ml and the samples were mixed for 12 hours at 4°C. The samples were then dialyzed against 0.1 M sodium acetate, pH 5.0, and used for immunization of rabbits. The resulting antisera were affinity purified on Affigel (Bio-Rad) columns to which the corresponding peptides had been coupled. Using 50 mM glycine, pH 3.0, for elution, fractions containing specific antibodies were collected and neutralized by addition of 1 M Tris, pH 7.4, followed by extensive dialysis against phosphate buffered saline (PBS). Antibodies were concentrated using Centricon concentrator spin columns, cut off 10 kDa (Amicon), and sterile filtered. Immunoglobulin fractions from preimmune sera were isolated on Protein A-Sepharose columns and thereafter eluted and dialyzed as the antisera.

Radioactive labeling and immunoprecipitation

Porcine aortic endothelial (PAE) cells expressing wild-type PDGF β-receptors (PAE/PDGFR-β) were labeled in cysteine- and methionine-free MCDB 104 medium containing 100 μCi/ml each of [^{35}S]cysteine and [^{35}S]methionine (Amersham Pharmacia Biotech) for 3 hours at 37°C. Labeled cells were washed with ice-cold PBS and lysed in a Triton-based lysis buffer (0.5% Triton X-100, 0.5% deoxycholate (Merck), 20 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 10 mM EDTA, 1% Trasylol (Bayer) and 1 mM phenylmethylsulfonyl fluoride (Sigma)). The lysates were centrifuged at 10000 ***g*** for 15 minutes and the supernatants were incubated with p110α or p110β preimmune serum or affinity purified antiserum for 2 hours at 4°C. In indicated cases the antiserum was incubated with 200 μM of the peptide used for immunization at 37°C for 30 minutes prior to addition of cell lysate. The immune-complexes were bound to Protein A-Sepharose by incubation for 30 minutes at 4°C. The beads were washed three times with lysis buffer and 3 times with high salt buffer (0.5% Triton X-100, 0.5% deoxycholate, 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 10 mM EDTA), and once in distilled water. The immune complexes were eluted from the beads by boiling for 3 minutes in sample buffer (4% SDS, 0.2 M Tris-HCl pH 8.0, 0.5 M sucrose, 5 mM EDTA, 0.01% bromophenol blue (Merck), 3% β-mercaptoethanol) and analyzed by SDS-gel electrophoresis using 7% polyacrylamide gels, followed by fluorography and exposure to film.

Western blotting

PAE cells expressing wild-type PDGF β-receptors were grown to subconfluence in Ham's F12 medium supplemented with 10% FCS. Thereafter, the cells were washed with ice-cold PBS containing 100 μM orthovanadate, and lysed in Nonidet P-40 (NP-40, United States Biochemical) lysis buffer (1% NP-40, 20 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Trasylol and 1 mM phenylmethylsulfonyl fluoride) on ice for 10 minutes. Clarified cell lysates were incubated with p110α or p110β antiserum in the presence and absence of blocking peptide as described above, and immune complexes were separated by SDS-gel electrophoresis. Proteins were transferred to Hybond C extra filters (Amersham Pharmacia Biotech) using electrotransfer (Trans-Blot Cell; Bio-Rad, Hercules, CA) and blotted with antiserum against p110α or p110β, or monoclonal antibodies to phosphotyrosine (PY20).

PI3-K assay

Recombinant proteins p85α/p110β and p85α/p110α were expressed in baculovirus infected cells (B. Vanhaesebroeck et al., unpublished data), and immobilized on the (p)YVPMLG (pY=phosphorylated tyrosine) peptide coupled to Actigel ALD Superflow (Sterogene), washed in kinase buffer (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5 mM EGTA) and incubated for 30 minutes at room temperature in the absence or presence of affinity purified peptide antibodies or Protein A-Sepharose purified antibodies from preimmune sera. Following addition of sonicated phosphatidylinositol (10 μg), Mg^{2+} (10 mM), ATP (1 mM), and [γ^{32}P]ATP (10 μCi), the lipid kinase assay was performed for 10 minutes at 37°C. The lipid products were separated by thin layer chromatography and detected by autoradiography. The amount of ^{32}P activity in each spot was quantified using a bioimager (Fuji).

Microinjection

PAE/PDGFR-β cells cultured on coverslips in Ham's F12 medium supplemented with 10% FCS were serum-starved overnight and microinjected with affinity purified antibodies at final concentrations of 1-2 mg/ml or PBS together with either lysine fixable, dextran-conjugated fluorescein 10,000 MW, or lysine fixable, dextran-conjugated Oregon Green 10,000 MW (Molecular Probes) at final concentrations of 0.05%. Eppendorf microinjection devices were used with a constant pressure of about 50 hPa, injection pressure of 100 hPa and an injection time of 0.1-0.2 seconds. The cells were then returned to the incubator to recover for 20-30 minutes before further treatment. For PDGF-stimulation purposes, 181 cells were microinjected with antibodies against p110α, 176 cells with antibodies against p110β and 147 cells with PBS. For insulin-stimulation purposes, 257 cells were microinjected with antibodies against p110α, 242 cells with antibodies against p110β and 217 cells with PBS. Approximately 60 cells were microinjected per coverslip in each case.

Visualization of filamentous actin

Microinjected cells were treated with 100 ng/ml PDGF-BB for 5 minutes at 37°C or 1 μM insulin for 8 minutes at 37°C before fixation in 2% formaldehyde. Control cells were fixed directly after recovery. After fixation the cells were permeabilized with 0.1% Triton X-100

in PBS, and incubated with TRITC-conjugated phalloidin (0.6 μM; Sigma) for 10-60 minutes before mounting.

RESULTS

The antibodies raised against p110α and p110β are isoform specific

To analyze the specific roles of p110α and p110β PI3-K in PDGF receptor mediated signaling, peptide antisera were raised against the extreme C-termini of the p110 isoforms, as described in Materials and Methods. The ability of the affinity purified antibodies to precipitate the respective p110 isoforms was investigated using lysates of ^{35}S-labelled PAE/PDGFR-β cells. Both the p110α and p110β antibodies precipitated components of 110 and 85 kDa that were not recognized by the preimmune sera and which could be competed out upon preincubation of the antiserum with the corresponding immunizing peptides (Fig. 1). The 85 kDa component comigrated with a component precipitated by an antiserum against the p85 subunit of PI3-K (data not shown). These data indicate that the antibodies against p110α and p110β both recognize components of the expected size of 110 kDa, which most likely are associated with the regulatory p85 subunit of PI3-K. In addition, several other components of 160, 125 and 92 kDa were precipitated with both the p110α and p110β antibodies which could be efficiently competed out in the presence of inhibitory peptides. We have not further identified these proteins but they may represent splice variants of the p110 subunits, related proteins or proteins which associate with the p85 and/or the p110 subunits.

To characterize the specificity of the p110 antisera, lysates from unstimulated or PDGF-BB-stimulated PAE/PDGFR-β cells, were incubated with either preimmune sera or antisera against p110α or p110β in the presence and absence of the peptides used for immunization. The resulting immune complexes were analyzed by immunoblotting with monoclonal antibodies to phosphotyrosine (PY20), and subsequently with antisera against p110α or p110β. As seen in Fig. 2, the p110α antiserum precipitated p110α, which in PDGF-stimulated cells formed a complex with the PDGF β-receptor, as detected by the anti-phosphotyrosine antibodies. Neither p110α, nor the 190 kDa PDGF β-receptor were detected by the preimmune antiserum, or when the p110α antiserum was preincubated with the peptide used for immunization. When using anti-p110α antiserum in the subsequent blotting of the same filter, bands corresponding to p110α were detected only when using immune, unblocked serum. Immunoblotting with the antiserum against p110β revealed no specific reactivity with the p110α immunoprecipitates.

Immunoprecipitation using the p110β antiserum showed complex-formation between p110β and the PDGFβ-receptor in PDGF-BB treated cells, as detected by the anti-phosphotyrosine antibodies. Precipitation with preimmune serum, or blocked p110β antiserum, followed by immunoblotting with PY20, failed to visualize the PDGF β-receptor or p110β. There was no reactivity in the subsequent immunoblottings with either p110α or p110β antisera.

These data show that both antisera against p110α and p110β work well in immunoprecipitation and that both p110 subunits

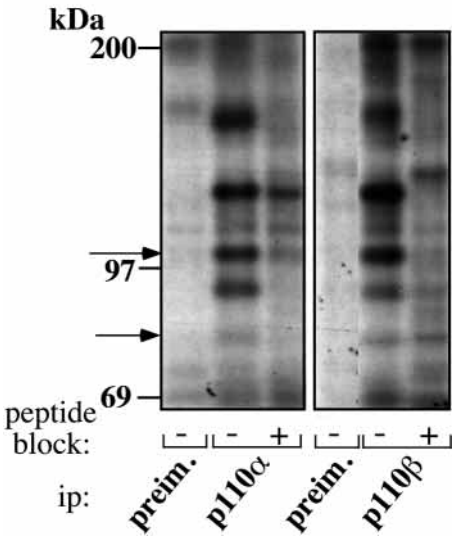

Fig. 1. Immunoprecipitation using affinity purified antibodies against p110α and p110β. PAE/PDGFR-β cells were labeled with [^{35}S]cysteine and [^{35}S]methionine. The lysates were incubated with p110α or p110β preimmune sera or affinity purified antibodies in the presence or absence of blocking peptide, as indicated, and the immune complexes were analyzed by SDS-gel electrophoresis and fluorography. Arrows indicate the migration position of p110 and p85. Markers of molecular masses (kDa) are indicated. ip, immunoprecipitation.

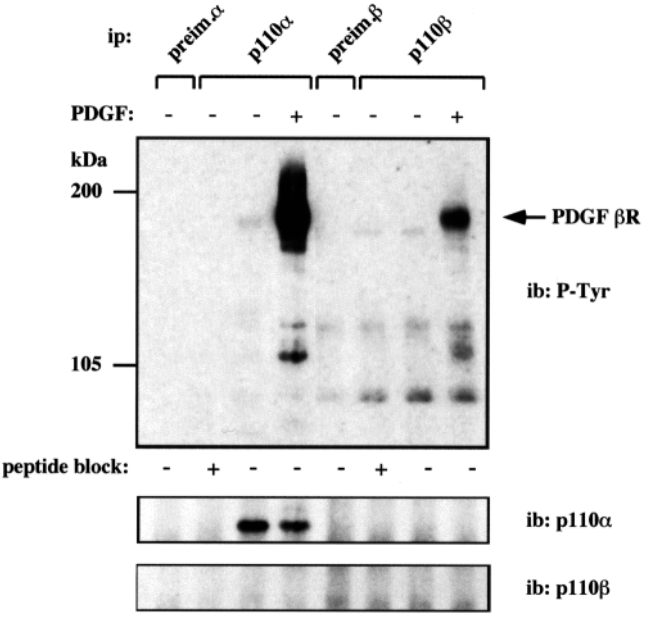

Fig. 2. The PDGF receptor associates with both p110α and p110β. PAE/PDGFR-β cell lysate from cells treated with PDGF-BB (+) or not (-) were incubated with either preimmune sera or antisera against p110α or p110β in the presence and absence of blocking peptide as indicated. The resulting immune complexes were separated by SDS-gel electrophoresis. Proteins were transferred to filters and probed with antibodies against phosphotyrosine, p110α or p110β, as indicated. The position of the PDGF β-receptor is indicated by an arrow. The markers of molecular masses (kDa) are indicated. ip, immunoprecipitation; ib, immunoblot.

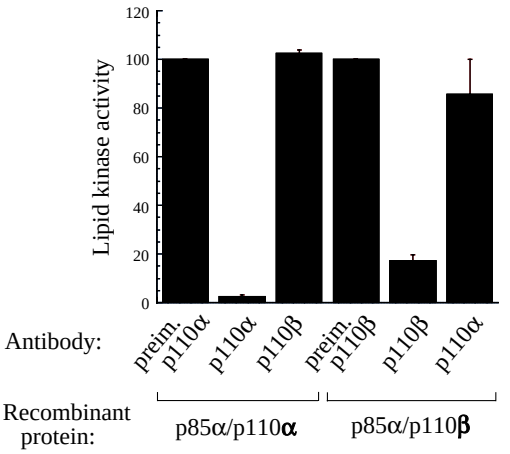

Fig. 3. The antibodies against p110α and p110β specifically inhibit the lipid kinase activity of the PI3-K isoform they were raised against. Recombinant p85α/p110α or p85α/p110β, bound to a peptide corresponding to the PI3-K binding motif of the PDGF β-receptor, were analyzed in a PI3-K assay in the presence of preimmune antisera or affinity-purified antibodies against p110α and p110β. The data are presented as percentage of PI3-K activity remaining after addition of antibodies, compared with activity obtained after addition of preimmune serum. PI3-K activity was determined by measuring the ^{32}P incorporated into the PI(3)P product using Bioimager analysis.

form complexes with the activated PDGF β-receptor. Moreover, whereas the p110β antiserum is unsuitable for blotting, the p110α antiserum reacts in an isoform-specific manner and detects p110α, but not p110β, in immunoblotting.

The p110α and p110β antibodies inhibit the lipid kinase activity of PI3-K

The inhibitory effect of the p110 antibodies on PI3-K activity was then investigated (Fig. 3). Recombinant p85α/p110α or p85α/p110β proteins, bound to a peptide corresponding to the Tyr751 PI3-K binding motif of the PDGF β-receptor, were analyzed in a PI3-K assay in the presence of preimmune serum or affinity purified antibodies. The p110α antibodies reduced the lipid kinase activity of the p110α PI3-K drastically, with only 1 to 4% of the activity remaining. The p110β antibodies lowered the lipid kinase activity down to about 17% of the value obtained after incubation with preimmune serum. The antisera showed specificity for the individual isoforms; the p110α antibodies had no effect on p110β activity, and the p110β antibodies had no effect on p110α activity.

Inhibition of p110α, but not p110β, affects PDGF-mediated actin reorganization in PAE cells

The inhibitory effect of the antibodies provided tools to study the involvement of p110α and p110β in biological responses in PAE cells. We first studied PDGF-induced actin reorganization which has been reported to be mediated via PI3-K-dependent pathways (Wennström et al., 1994a,b). PAE/PDGFR-β cells seeded onto coverslips were microinjected with PBS or with antibodies against either of the α- or β-isoforms of p110. After recovery, the cells were treated with PDGF for 5 minutes, fixed and stained for filamentous actin with TRITC-conjugated phalloidin. The p110α antibodies inhibited both the formation of edge ruffles and the loss of stress fibers typically seen after PDGF stimulation of PAE/PDGFR-β (Wennström et al., 1994b; Fig. 4A,B). Of the non-injected cells, 72% (1078 of 1479) responded to the

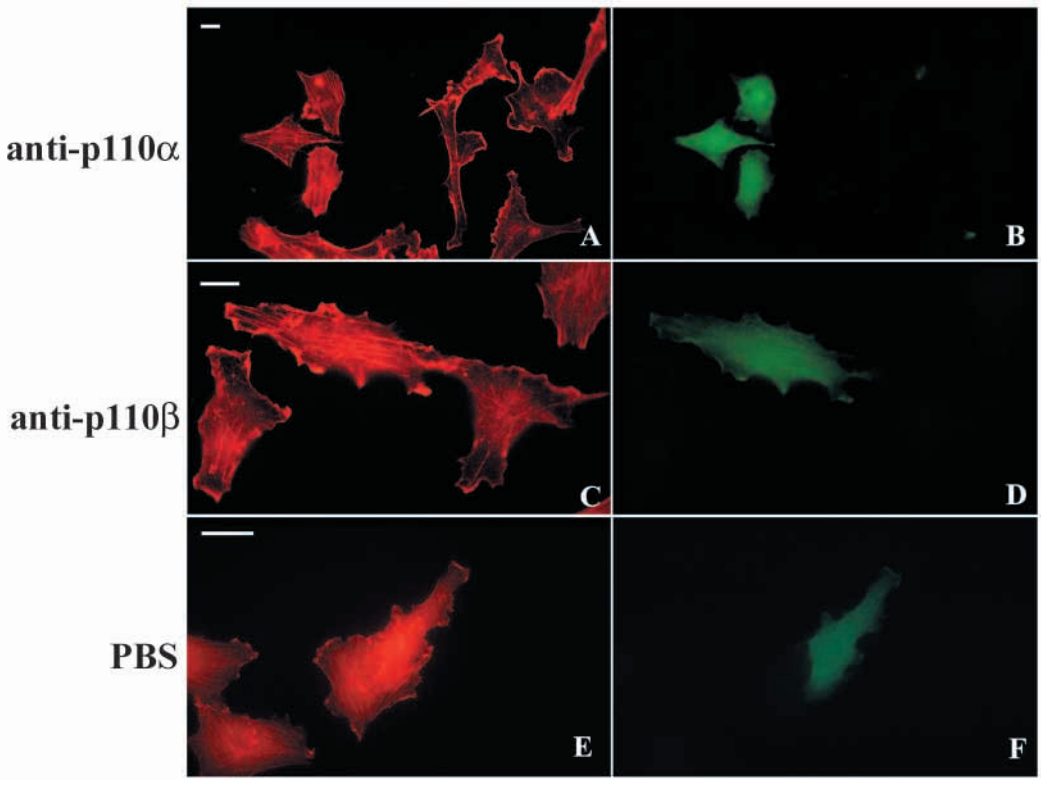

Fig. 4. Microfilament distribution in PAE/PDGFR-β after microinjection of antibodies against the α- and β-isoforms of p110. Twenty minutes after microinjection, cells were stimulated with 100 ng/ml PDGF-BB for five minutes followed by fixation and TRITC-conjugated phalloidin staining of filamentous actin (A,C,E). The green fluorescent marker (FITC-dextran; B,D,F) shows the injected cells. Cells which received antibodies against p110α (A,B) lack the typical edge-ruffles seen in cells injected with antibodies against p110β (C,D), or with PBS (E,F), and still display prominent stress fibers. Bars, 20 μm.

PDGF-exposure with typical changes of their microfilament distribution. In contrast, of 181 cells injected with p110α antibodies only 30% displayed PDGF-induced reorganization. Of 147 cells injected with PBS, 58% showed PDGF-induced actin reorganization i.e. a slightly lower fraction compared to non-injected cells; the difference may be due to mechanical damage of some cells during the microinjection. The p110β antibodies were injected into 176 cells, of which 55% showed a microfilament distribution typical for PDGF-stimulated cells. Therefore, in contrast to the results obtained with the p110α antibodies, the effect of the p110β antibodies (Fig. 4C,D) could not be distinguished from the effect seen after microinjection of PBS (Fig. 4E,F) or preimmune serum (data not shown). This suggests that the α-isoform of p110 is involved in signaling from the PDGF β-receptor to the microfilament system, while p110β is not essential for this pathway.

Inhibition of p110β, but not p110α affects insulin-mediated actin reorganization in PAE cells

Rearrangement of actin also takes place in many cell types after insulin stimulation (Shepherd et al., 1997) and results in ruffling and debundling similar to what is seen after PDGF stimulation. To study the involvement of the p110α and p110β isoforms in insulin-induced actin dynamics, p110α and p110β antibodies were microinjected into PAE/PDGFR-β cells that were then stimulated with insulin for 8 minutes at 37°C (Fig. 5). After recovery, filamentous actin was visualized, as described above. In this case, inhibition of p110β (Fig. 5C,D) led to loss of ruffling activity induced by insulin in approximately 70% of microinjected cells, whereas only approximately 20% of microinjected cells showed decreased edge ruffling when microinjected with antibodies against p110α (Fig. 5A,B) or PBS (Fig. 5E,F). Microinjection of p110β antibodies caused a decrease but not a complete loss of stress fibres (Fig. 5C). Therefore, it appears that the effect of insulin stimulation on the actin organization in these cells is dependent on the β-isoform of p110. Together these findings indicate a differential involvement of p110α and p110β in PAE/PDGFR-β cells with respect to actin reorganization depending on which of the two growth factor receptors that is activated.

DISCUSSION

Lipids phosphorylated at the 3′ position of the inositol ring have been shown to have important roles in signal transduction pathways downstream of almost every activated receptor complex involving tyrosine kinases and some heterotrimeric G-proteins (Fry, 1994; Stephens et al., 1993). In particular, PI3-K dependent processes have been shown to cause reorganization of the microfilament system (Wennström et al., 1994a,b). In this study we have investigated the roles of two of the most widely expressed p110 catalytic subunits of the PI3-K, namely p110α and p110β, in mediating PDGF- and insulin-induced actin reorganization. By microinjecting specific, inhibitory antibodies towards either p110α or p110β we have been able to disrupt the signaling capacity of one isoform without disturbing the other, and thereby identified unique pathways in which the isoforms are involved. Our data suggest that PDGF- and insulin-induced membrane ruffling are maintained by differential involvement of p110α and p110β, such that p110α but not p110β is important for PDGF-induced rearrangement of the cytoskeleton, whereas p110β but not p110α is important for insulin-induced actin reorganization.

In the submembraneous space of eukaryotic cells the microfilament system forms a dense and highly ordered arrangement of actin filaments and accessory proteins (Höglund et al., 1980; Small, 1981; Small et al., 1999). The dynamics of the actin filament arrangement, controlled by actin binding proteins like profilin, cofilin and gelsolin (Ayscough, 1998), causes the rapid changes in cell morphology that activated cell surface receptors give rise to (Chinkers et al., 1979; Mellström et al., 1983) and appears to be closely associated with polyphosphoinositide turn-over (Cantrell, 1998; Hartwig et al., 1995). In vitro studies have shown that profilin influences PLCγ-induced hydrolysis of $PI(4,5)P_2$ (Goldschmidt-Clermont et al., 1990, 1991), dissociates from actin upon binding of polyphosphoinositides (Lassing and Lindberg, 1985; Lu et al., 1996), and stimulates PI3-K activity (Singh et al., 1996).

There are previous observations suggesting that the PI3-K isoforms have distinct functions; the p110α subunit has been shown to be involved in PDGF-, insulin- and EGF-mediated, but not in bombesin- or lysophosphatidic acid (LPA)-mediated, mitogenic responses (Roche et al., 1994). Recently it has been shown that p110β is necessary for insulin- and LPA-mediated,

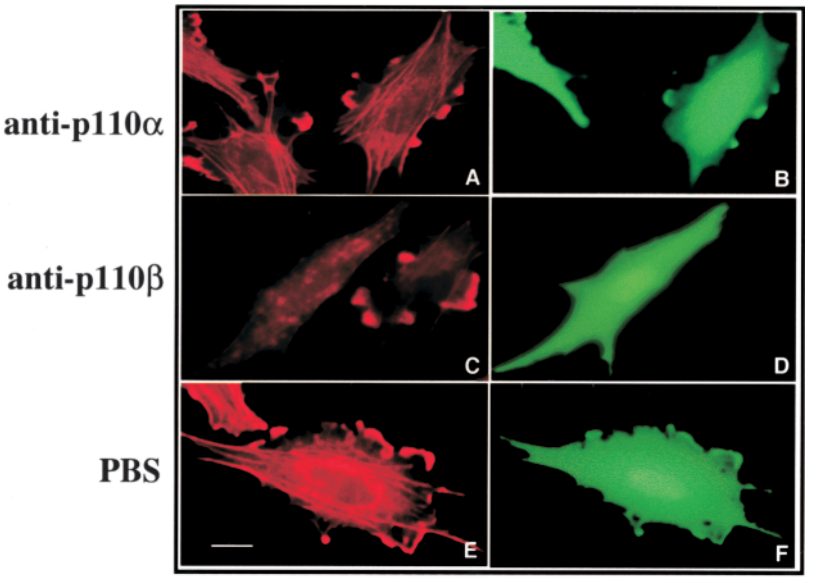

Fig. 5. Microinjection of p110β antibodies but not p110α antibodies affects insulin-mediated actin reorganization in PAE cells. PAE/PDGFR-β cells seeded onto coverslips were microinjected with either p110α antibodies, p110β antibodies or with PBS, and left to recover for 20-30 minutes. Cells were then treated with insulin for 8 minutes at 37°C and thereafter fixed and stained with TRITC-conjugated phalloidin to visualize the actin cytoskeleton. The green fluorescent marker (Oregon Green-dextran; B,D,F) indicates injected cells. Cells which received antibodies against p110β (C,D) lack the typical edge-ruffles seen in cells injected with antibodies against p110α (A,B), or with PBS (E,F). Bar, 20 μm.

but not PDGF-mediated, mitogenic responses (Roche et al., 1998). In contrast to our results, Siddhanta et al. (1998) have shown that microinjection of p110α antibodies blocks insulin-stimulated membrane ruffling. This apparent discrepancy may in part be due to the use of different cell lines; the cells used by Siddhanta et al. (1998) overexpress the insulin receptor whereas the PAE cells used in this study express endogenous levels of the insulin receptor. Recently, it has been shown that CSF1-induced DNA synthesis is dependent on p110α activity but not on p110β or p110δ activity. Conversely, p110β and p110δ, but not p110α, are involved in CSF1-induced actin reorganization and migration (Vanhaesebroeck et al., 1999a). In addition, the expression level of the p85α adapter subunit decreased in response to metabolic changes, whereas those of the adapter proteins p50 and p55 increased (Kerouz et al., 1997). There are also data indicating insulin-induced association of p110β (but not p110α) with glucose-transporter containing compartments (Wang et al., 1998). Furthermore, p110β and p110δ, but not p110α are recruited to the plasma membrane upon redox activation of Ras (Deora et al., 1998), and Rab5 binds human Vps34 and p110β, but not p110α (Christoforidis et al., 1999). There is therefore increasing evidence that different PI3-K isoforms have different roles in signaling.

The mechanisms whereby specific PI3-K isoforms preferentially becomes involved in different signaling pathways still remains to be elucidated. It is possible that the activated PDGF and insulin receptors are differentially compartmentalized together with the specific p110 isoforms that mediate their effects. There are data showing that the diverse responses mediated by PDGF- versus insulin-induced PI3-K activity are associated with differences in subcellular localization of the respective growth factor-receptors (Corvera and Czech, 1998; Nave et al., 1996). However, this does not explain the reason for the involvement of one PI3-K isoform, rather than the other, in specific growth factor-mediated changes of actin organization. There are no apparent difference in the subcellular localization of two of the PI3-K isoforms, p110α and p110δ, in macrophages (C. Wells, B. Vanhaesebroeck and A. J. Ridley unpublished data). It is, however, not known whether p110α and p110β are differentially localized. Selective recruitment of PI3-K p110 isoforms has been excluded in studies involving the receptors for CSF-1, c-kit, IL3, GM-CSF and CD28 (Vanhaesebroeck et al., 1997b, 1999a). Despite this, CD28 stimulation of Jurkat cells has opposing effects on the lipid kinase activity of p110β and p110δ (Vanhaesebroeck et al., 1999b) indicating that growth factor receptors and other signals downstream of these receptors, may induce specific p110 isoform responses. Another potential explanation for the differences in involvement of the PI3-K catalytic isoforms is differential interaction with the regulatory adapter proteins. However, there are no indications of differential association between the regulatory subunit isoforms p85α and β, and the catalytic subunit isoforms p110α, β and δ (Vanhaesebroeck et al., 1997a) although the relative in vivo affinities of these interactions are not known. There are data suggesting that the adapter proteins associate with insulin-induced signaling complexes in the relative order of p50>p85α>p55α (Antonetti et al., 1996; Inukai et al., 1997; Shepherd et al., 1997). Examples of p85 binding proteins, besides the catalytic subunits, are the actin binding proteins profilin and α-actinin, the adaptor Grb2, the proto-oncogene Cbl, the tyrosine kinase $p125^{FAK}$ and Src family members of tyrosine kinases (Guinebault et al., 1995; Hunter et al., 1997; Liu et al., 1993; Pleiman et al., 1994; Shibasaki et al., 1994; Singh et al., 1996; Wang et al., 1995; Yuan et al., 1997). Different p85 adapter proteins and splice variants of these can bind to specific subsets of downstream elements thereby contributing to specific cellular responses.

In conclusion, this paper illustrates that the functions of the catalytic p110α and p110β subunits during PDGF and insulin stimulation are not fully redundant. Rather, each isoform appears to be specifically engaged in mediating changes in actin reorganization induced by one receptor but not the other.

This work was partly supported by Axel and Margaret Ax:son Johnsons Foundation (Carl-Henrik Heldin), the Swedish Natural Research Council (Roger Karlsson) and by the Swedish Cancer Foundation (Lena Claesson-Welsh). Bart Vanhaesebroeck is supported in part by the Flemish Fund for Scientific Research, Belgium.

REFERENCES

Antonetti, D. A., Algenstaedt, P. and Kahn, C. R. (1996). Insulin receptor substrate 1 binds two novel splice variants of the regulatory subunit of phosphatidylinositol 3-kinase in muscle and brain. *Mol. Cell Biol.* **16**, 2195-2203.

Ayscough, K. R. (1998). In vivo functions of actin-binding proteins. *Curr. Opin. Cell Biol.* **10**, 102-111.

Cantrell, D. (1998). Lymphocyte signalling: a coordinating role for Vav? *Curr. Biol.* **8**, R535-538.

Carpenter, C. L., Auger, K. R., Chanudhuri, M., Yoakim, M., Schaffhausen, B., Shoelson, S. and Cantley, L. C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. *J. Biol. Chem.* **268**, 9478-9483.

Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J. and Kahn, C. R. (1994). Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. *Mol. Cell Biol.* **14**, 4902-4911.

Chinkers, M., McKanna, J. A. and Cohen, S. (1979). Rapid induction of morphological changes in human carcinoma cells A-431 by epidermal growth factors. *J. Cell Biol.* **83**, 260-265.

Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S., Waterfield, M. D., Backer, J. M. and Zerial, M. (1999). Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nature Cell Biol.* **1**, 249-252.

Claesson-Welsh, L. (1994). Platelet-derived growth factor receptor signals. *J. Biol. Chem.* **269**, 32023-6.

Cohen, G. B., Ren, R. and Baltimore, D. (1995). Modular binding domains in signal transduction proteins. *Cell* **80**, 237-248.

Corvera, S. and Czech, M. P. (1998). Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. *Trends Cell Biol.* **8**, 442-446.

Deora, A. A., Win, T., Vanhaesebroeck, B. and Lander, H. M. (1998). A Redox-triggered Ras-Effector Interaction. Recruitment of phosphatidylinositol 3′-kinase to ras by redox stress. *J. Biol. Chem.* **273**, 29923-29928.

Fantl, W. J., Escobedo, J. A., Martin, G. A., Turck, C. W., del Rosario, M., McCormick, F. and Williams, L. T. (1992). Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. *Cell* **69**, 413-423.

Frevert, E. U. and Kahn, B. B. (1997). Differential effects of constitutively active phosphatidylinositol 3-kinase on glucose transport, glycogen synthase activity, and DNA synthesis in 3T3-L1 adipocytes. *Mol. Cell Biol.* **17**, 190-198.

Fry, M. J. (1994). Structure, regulation and function of phosphoinositide 3-kinases. *Biochim. Biophys. Acta* **1226**, 237-268.

Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J. and

Pollard, T. D. (1990). The actin-binding protein profilin binds to PIP2 and inhibits its hydrolysis by phospholipase C. *Science* **247**, 1575-1578.

Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G. and Pollard, T. D. (1991). Regulation of phospholipase C-gamma 1 by profilin and tyrosine phosphorylation. *Science* **251**, 1231-1233.

Guinebault, C., Payrastre, B., Racaud-Sultan, C., Mazarguil, H., Breton, M., Mauco, G., Plantavid, M. and Chap, H. (1995). Integrin-dependent translocation of phosphoinositide 3-kinase to the cytoskeleton of thrombin-activated platelets involves specific interactions of p85α with actin filaments and focal adhesion kinase. *J. Cell Biol.* **129**, 831-842.

Hartwig, J. H., Bokoch, G. M., Carpenter, C. L., Janmey, P. A., Taylor, L. A., Toker, A. and Stossel, T. P. (1995). Thrombin receptor ligation and activated Rac uncap actin filament barbed ends through phosphoinositide synthesis in permeabilized human platelets. *Cell* **82**, 643-653.

Heldin, C. H., Östman, A. and Rönnstrand, L. (1998). Signal transduction via platelet-derived growth factor receptors. *Biochim. Biophys. Acta* **1378**, F79-113.

Hiles, I. D., Otsu, M., Volinia, S., Fry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N. F., Hsuan, J. J., Courtneidge, S. A., Parker, P. J. and Waterfield, M. D. (1992). Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell* **70**, 419-429.

Höglund, A. S., Karlsson, R., Arro, E., Fredriksson, B. A. and Lindberg, U. (1980). Visualization of the peripheral weave of microfilaments in glia cells. *J. Muscle Res. Cell Motil.* **1**, 127-146.

Hooshmand-Rad, R., Claesson-Welsh, L., Wennström, S., Yokote, K., Siegbahn, A. and Heldin, C. H. (1997). Involvement of phosphatidylinositide 3′-kinase and Rac in platelet-derived growth factor-induced actin reorganization and chemotaxis. *Exp. Cell Res.* **234**, 434-441.

Hu, P., Mondino, A., Skolnik, E. Y. and Schlessinger, J. (1993). Cloning of a novel, ubiquitously expressed human phosphatidylinositol 3-kinase and identification of its binding site on p85. *Mol. Cell Biol.* **13**, 7677-7688.

Hunter, S., Koch, B. L. and Anderson, S. M. (1997). Phosphorylation of cbl after stimulation of Nb2 cells with prolactin and its association with phosphatidylinositol 3-kinase. *Mol. Endocrinol.* **11**, 1213-1222.

Inukai, K., Funaki, M., Ogihara, T., Katagiri, H., Kanda, A., Anai, M., Fukushima, Y., Hosaka, T., Suzuki, M., Shin, B. C., Takata, K., Yazaki, Y., Kikuchi, M., Oka, Y. and Asano, T. (1997). p85α gene generates three isoforms of regulatory subunit for phosphatidylinositol 3-kinase (PI 3-Kinase), p50α, p55α, and p85α, with different PI 3-kinase activity elevating responses to insulin. *J. Biol. Chem.* **272**, 7873-7882.

Jhun, B. H., Rose, D. W., Seely, B. L., Rameh, L., Cantley, L., Saltiel, A. R. and Olefsky, J. M. (1994). Microinjection of the SH2 domain of the 85-kilodalton subunit of phosphatidylinositol 3-kinase inhibits insulin-induced DNA synthesis and c-fos expression. *Mol. Cell Biol.* **14**, 7466-7475.

Kaliman, P., Canicio, J., Shepherd, P. R., Beeton, C. A., Testar, X., Palacin, M. and Zorzano, A. (1998). Insulin-like growth factors require phosphatidylinositol 3-kinase to signal myogenesis: dominant negative p85 expression blocks differentiation of L6E9 muscle cells. *Mol. Endocrinol*. **12**, 66-77.

Kazlauskas, A., Kashishian, A., Cooper, J. A. and Valius, M. (1992). GTPase-activating protein and phosphatidylinositol 3-kinase bind to distinct regions of the platelet-derived growth factor receptor beta subunit. *Mol. Cell Biol.* **12**, 2534-2544.

Kerouz, N. J., Horsch, D., Pons, S. and Kahn, C. R. (1997). Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. *J. Clin. Invest.* **100**, 3164-3172.

Klippel, A., Escobedo, J. A., Hirano, M. and Williams, L. T. (1994). The interaction of small domains between the subunits of phosphatidylinositol 3-kinase determines enzyme activity. *Mol. Cell Biol.* **14**, 2675-2685.

Kotani, K., Yonezawa, K., Hara, K., Ueda, H., Kitamura, Y., Sakaue, H., Ando, A., Chavanieu, A., Calas, B., Grigorescu, F., Nishiyama, M., Waterfield, M. D. and Kasuga, M. (1994). Involvement of phosphoinositide 3-kinase in insulin- or IGF-induced membrane ruffling. *EMBO J.* **13**, 2313-2321.

Kundra, V., Escobedo, J. A., Kazlauskas, A., Kim, H. K., Rhee, S. G., Williams, L. T. and Zetter, B. R. (1994). Regulation of chemotaxis by the platelet-derived growth factor receptor-beta. *Nature* **367**, 474-476.

Kurosu, H., Maehama, T., Okada, T., Yamamoto, T., Hoshino, S., Fukui, Y., Ui, M., Hazeki, O. and Katada, T. (1997). Heterodimeric phosphoinositide 3-kinase consisting of p85 and p110β is synergistically activated by the βγ subunits of G proteins and phosphotyrosyl peptide. *J. Biol. Chem.* **272**, 24252-24256.

Lassing, I. and Lindberg, U. (1985). Specific interaction between phosphatidylinositol 4, 5-bisphosphate and profilactin. *Nature* **314**, 472-474.

Liu, X., Marengere, L. E., Koch, C. A. and Pawson, T. (1993). The v-Src SH3 domain binds phosphatidylinositol 3′-kinase. *Mol. Cell Biol.* **13**, 5225-5232.

Lu, P. J., Shieh, W. R., Rhee, S. G., Yin, H. L. and Chen, C. S. (1996). Lipid products of phosphoinositide 3-kinase bind human profilin with high affinity. *Biochemistry* **35**, 14027-14034.

McIlroy, J., Chen, D., Wjasow, C., Michaeli, T. and Backer, J. M. (1997). Specific activation of p85-p110 phosphatidylinositol 3′-kinase stimulates DNA synthesis by ras- and p70 S6 kinase-dependent pathways. *Mol. Cell Biol.* **17**, 248-255.

Mellström, K., Höglund, A. S., Nistér, M., Heldin, C. H., Westermark, B. and Lindberg, U. (1983). the effect of platelet-derived growth factor on morphology and motility of human glial cells. *J. Muscle Res. Cell Motil.* **4**, 589-609.

Nave, B. T., Haigh, R. J., Hayward, A. C., Siddle, K. and Shepherd, P. R. (1996). Compartment-specific regulation of phosphoinositide 3-kinase by platelet-derived growth factor and insulin in 3T3-L1 adipocytes. *Biochem. J.* **318**, 55-60.

Nobes, C. D., Hawkins, P., Stephens, L. and Hall, A. (1995). Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J. Cell Sci.* **108**, 225-233.

Pleiman, C. M., Hertz, W. M. and Cambier, J. C. (1994). Activation of phosphatidylinositol-3′ kinase by Src-family kinase SH3 binding to the p85 subunit. *Science* **263**, 1609-1612.

Roche, S., Koegl, M. and Courtneidge, S. A. (1994). The phosphatidylinositol 3-kinase α is required for DNA synthesis induced by some, but not all, growth factors. *Proc. Nat. Acad. Sci. USA* **91**, 9185-9189.

Roche, S., Downward, J., Raynal, P. and Courtneidge, S. A. (1998). A function for phosphatidylinositol 3-kinase beta (p85α-p110β) in fibroblasts during mitogenesis: requirement for insulin- and lysophosphatidic acid-mediated signal transduction. *Mol. Cell Biol.* **18**, 7119-7129.

Shepherd, P. R., Nave, B. T., Rincon, J., Nolte, L. A., Bevan, A. P., Siddle, K., Zierath, J. R. and Wallberg-Henriksson, H. (1997). Differential regulation of phosphoinositide 3-kinase adapter subunit variants by insulin in human skeletal muscle. *J. Biol. Chem.* **272**, 19000-19007.

Shibasaki, F., Fukami, K., Fukui, Y. and Takenawa, T. (1994). Phosphatidylinositol 3-kinase binds to α-actinin through the p85 subunit. *Biochem. J.* **302**, 551-557.

Siddhanta, U., McIlroy, J., Shah, A., Zhang, Y. and Backer, J. M. (1998). Distinct roles for the p110α and hVPS34 phosphatidylinositol 3′-kinases in vesicular trafficking, regulation of the actin cytoskeleton, and mitogenesis. *J. Cell Biol.* **143**, 1647-1659.

Singh, S. S., Chauhan, A., Murakami, N. and Chauhan, V. P. (1996). Profilin and gelsolin stimulate phosphatidylinositol 3-kinase activity. *Biochemistry* **35**, 16544-16549.

Small, J. V. (1981). Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of actin meshworks. *J. Cell Biol.* **91**, 695-705.

Small, J. V., Rottner, K. and Kaverina, I. (1999). Functional design in the actin cytoskeleton. *Curr. Opin. Cell Biol.* **11**, 54-60.

Stephens, L. R., Jackson, T. R. and Hawkins, P. T. (1993). Agonist-stimulated synthesis of phosphatidylinositol(3,4,5)-trisphosphate: a new intracellular signalling system? *Biochim. Biophys. Acta* **1179**, 27-75.

Stephens, L., Smrcka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C. and Hawkins, P. T. (1994). A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein βγ subunits. *Cell* **77**, 83-93.

Stoyanov, B., Volinia, S., Hanck, T., Rubio, I., Loubtchenkov, M., Malek, D., Stoyanova, S., Vanhaesebroeck, B., Dhand, R., Nurnberg, B., Gierschik, P., Seedorf, K., Hsuan, J. J., Waterfield, M. D. and Wetzker, R. (1995). Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. *Science* **269**, 690-693.

Tomiyama, K., Nakata, H., Sasa, H., Arimura, S., Nishio, E. and Watanabe, Y. (1995). Wortmannin, a specific phosphatidylinositol 3-kinase inhibitor, inhibits adipocytic differentiation of 3T3-L1 cells. *Biochem. Biophys Res. Commun*. **212**, 263-269.

Vanhaesebroeck, B., Leevers, S. J., Panayotou, G. and Waterfield, M. D. (1997a). Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem. Sci.* **22**, 267-272.

Vanhaesebroeck, B., Welham, M. J., Kotani, K., Stein, R., Warne, P. H., Zvelebil, M. J., Higashi, K., Volinia, S., Downward, J. and Waterfield, M. D. (1997b). P110δ, a novel phosphoinositide 3-kinase in leukocytes. *Proc. Nat. Acad. Sci. USA* **94**, 4330-4335.

Vanhaesebroeck, B., Jones, G. E., Allen, W. E., Zicha, D., Hooshmand-Rad, R., Sawyer, C., Wells, C., Waterfield, M. D. and Ridley, A. J. (1999a). Distinct PI(3)Ks mediate mitogenic signalling and cell migration in macrophages. *Nature Cell Biol.* **1**, 69-71.

Vanhaesebroeck, B., Higashi, K., Raven, C., Welham, M., Anderson, S., Brennan, P., Ward, S. G. and Waterfield, M. D. (1999b). Autophosphorylation of p110δ phosphoinositide 3-kinase: a new paradigm for the regulation of lipid kinases in vitro and in vivo. *EMBO J.* **18**, 1292-1302.

Wang, J., Auger, K. R., Jarvis, L., Shi, Y. and Roberts, T. M. (1995). Direct association of Grb2 with the p85 subunit of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **270**, 12774-12780.

Wang, Q., Bilan, P. J., Tsakiridis, T., Hinek, A. and Klip, A. (1998). Actin filaments participate in the relocalization of phosphatidylinositol 3-kinase to glucose transporter-containing compartments and in the stimulation of glucose uptake in 3T3-L1 adipocytes. *Biochem. J.* **331**, 917-928.

Wennström, S., Hawkins, P., Cooke, F., Hara, K., Yonezawa, K., Kasuga, M., Jackson, T., Claesson-Welsh, L. and Stephens, L. (1994a). Activation of phosphoinositide 3-kinase is required for PDGF-stimulated membrane ruffling. *Curr. Biol.* **4**, 385-393.

Wennström, S., Siegbahn, A., Yokote, K., Arvidsson, A. K., Heldin, C. H., Mori, S. and Claesson-Welsh, L. (1994b). Membrane ruffling and chemotaxis transduced by the PDGF β-receptor require the binding site for phosphatidylinositol 3′ kinase. *Oncogene* **9**, 651-660.

Yao, R. and Cooper, G. M. (1995). Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* **267**, 2003-2006.

Yuan, Z. M., Utsugisawa, T., Huang, Y., Ishiko, T., Nakada, S., Kharbanda, S., Weichselbaum, R. and Kufe, D. (1997). Inhibition of phosphatidylinositol 3-kinase by c-Abl in the genotoxic stress response. *J. Biol. Chem.* **272**, 23485-23488.